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REVIEW ARTICLE

Adolescent idiopathic scoliosis: evidence for intrinsic factors driving aetiology and progression

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Abstract Adolescent idiopathic scoliosis (AIS) is now considered to be a multifactorial heterogeneous disease, with recent genomic studies supporting the role of intrinsic factors in contributing to the onset of disease pathology and curve progression. Understanding the key molecular signalling pathways by which these intrinsic factors mediate AIS pathology may facilitate the development of pharmacological therapeutics and the identification of predictive markers of progression. The heterogenic nature of AIS has implicated multiple tissue types in the disease pathophysiology, including spinal bone, intervertebral disc and paraspinal muscles. In this review, we highlight some of the mechanisms and intrinsic molecular regulators within these different tissue types and review the evidence for their involvement in AIS pathology.

Keywords Adolescent idiopathic scolisosis · Epigenetics · Bone · Intervertebral disc · Paraspinal muscles

Introduction

Lateral curvature of the spine, for which there is no known cause, is the most common paediatric spinal deformity. In children aged between ten and 18 years, it is termed adolescent idiopathic scoliosis (AIS) and affects between 2 and 3 % of this population [42]. AIS is a highly heterogeneous condition, with some patients exhibiting rapidly progressive aggressive curves and others progressing more slowly with nonaggressive curves. AIS management includes bracing, growth modulation and fusion. However, these procedures are associated with significant morbidity [42, 49, 68].

A number of theories have been proposed regarding the cause of AIS, including metabolic [1] and biomechanical [21] hypotheses, and several tissue types have been implicated in its pathogenesis, including bone, intervertebral discs and paravertebral muscles. The consensus is therefore that AIS is a multifactorial disease [12], with increasing evidence from genetic studies for the central role of intrinsic factors in contributing to its pathology and progression. Ultimately, clarifying the molecular basis for these pathogenic drivers will facilitate the development of pharmacological therapeutics. Furthermore, it will help to identify predictive markers of aggressive curves to inform clinicians and patients regarding the likely success or failure of nonfusion modalities (bracing or tethering). This review focuses on evidence for the involvement of spinal bone, intervertebral disc and paraspinal muscles tissues in the pathophysiology of AIS and reviews some potential key mechanisms and intrinsic molecular regulators within these tissues and cell types.

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Intrinsic factors of the AIS spinal musculoskeletal system

Spinal bone

Importantly, there is now increasing evidence to support a role for abnormal spinal bone tissue as a primary intrinsic driver of AIS pathogenesis and a key determinant of curve progression

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[12, 15]. It is has previously been reported that AIS patients exhibit lower lumbar spine bone mineral density (LSBMD) [9, 10, 55] and altered vertebral growth [9, 10, 29], resulting in disparity between the growth of anterior and posterior vertebrae, resulting in rotational lordosis. Furthermore, recent studies have reported osteopaenia as a prognostic indicator of curve progression [23, 57]. Significantly, several studies have provided evidence for the dysregulation of key molecular signalling pathways that are known regulators of bone mass and osteoblast cellular function. It is well established that central to the regulation of bone mass is osteoblast expression of receptor activator of nuclear factor kappa-B ligand (RANKL), which stimulates osteoclast activity and bone resorption by binding to RANK on the osteoclast membrane, and the osteoblast expression of osteoprotegerin (OPG), which acts as a decoy receptor for RANKL, thus inhibiting osteoclast activity and bone resorption [30]. Critically, genetic studies have shown associations between LSBMD with polymorphisms of OPG in girls diagnosed with AIS [16]. Furthermore, increased serum concentration of RANKL and an increase in the RANKL:OPG ratio, which would promote greater bone resorption, have been reported in patients with AIS, and were found to be negatively correlated to LSBMD [55].

Another line of evidence to support the hypothesis that intrinsic factors are central to AIS spinal bone pathology comes from studies on melatonin signalling. Melatonin is believed to promote bone mass by increasing the differentiation of mesenchymal stem cells (MSCs) into osteoblasts [54], promoting osteoblast proliferation [40] and reducing osteoblast RANKL expression [28]. In animal models of idiopathic scoliosis, such as the chick experimental pinealectomy model, the development of idiopathic scoliosis-like changes has been associated with reduced levels of serum melatonin. Furthermore, administration of melatonin has been shown to prevent the development of scoliosis in both chick and rodent models [35, 36]. However, in humans, studies have predominantly reported no difference in serum melatonin levels in patients with or without AIS [5, 56], suggesting there would unlikely be any benefit to melatonin supplementation for patients with AIS. Critically, a more recent study has shown that melatonin can induce the proliferation of normal human osteoblasts but not osteoblasts from AIS female patients [37], suggesting an intrinsic dysfunction in melatonin signalling exists in human AIS spinal bone tissue. At present, the molecular basis for this intrinsic dysfunctional melatonin signalling is not fully understood. However, polymorphisms in melatonin receptor 1B have been associated with the occurrence of idiopathic scoliosis [46, 47]. Furthermore, it has been demonstrated that melatonin stimulation of AIS osteoblasts results in differential phosphorylation of the G inhibitory signalling proteins that are coupled to the melatonin receptors, compared with normal osteoblasts [3].

Similarly, recent data suggests that the known association of the hormone estrogen with the onset and development of AIS may also be due to intrinsic differences in AIS spinal bone. Importantly, studies have found no significant difference in circulating estrogen levels between patients with AIS compared with control individuals [48], but polymorphisms in estrogen receptors have been found to be associated with AIS susceptibility and curve severity [45, 69], suggesting altered intrinsic estrogen signalling.

Intervertebral disc and growth plate

Patients with AIS exhibit "wedging" of the intervertebral disc, which is associated with a shift in the position of the nucleus pulposus to the convexity of the curve [31]. It is currently unclear whether these changes are a secondary response to spinal curvature and altered loading. However, changes to the intervertebral disc in AIS have been reported to occur early in the disease process, as detected by differences in magnetic resonance imaging (MRI) signal intensity [17]. In addition, MRI studies have detected central as well as concave and convex vertebral growth plate abnormalities to be frequently located near the apex of the curve [14], which could indicate that these abnormalities are a primary event.

The biological processes and molecular mechanisms that underlie these observed growth plate intervertebral disc abnormalities are poorly understood. However, histopathological studies have provided evidence for the disorganisation of columns of chondrocytes in the convex zone of the growth plate from a patient with AIS [14]. Furthermore, several studies have provided evidence for intervertebral disc matrix degeneration in AIS, with reduced proteoglycan content [50], decreased sulphation and acetylation of proteoglycans within the cartilaginous end plate and nucleus pulposus [52]. In addition, in the annulus fibrosus of AIS patients, vacuolation [41] and abnormal localisation of collagen fibres have been reported [7].

Importantly, histological analysis comparing anterior and posterior AIS growth plates have shown larger proliferative and hypertrophic chondrocyte zones in anterior compared with posterior samples [70]. Similarly, significantly greater proliferative and apoptotic chondrocytes have been reported in the convex side than in the concave side in the apex vertebral growth plate in AIS [62]. It is known from studies of joint cartilage degeneration that proliferative hypertrophic chondrocytes display a differential phenotype. For example, reduced expression of type II collagen, increased expression of cartilaginous and aggrecan proteoglycan proteases and increased expression of transcription factor Runx2 and type 10 collagen [59], which ultimately facilitates cartilaginous matrix degeneration and endochondral ossification. Of relevance, therefore, is the finding that AIS convex and concave vertebral

growth plates display differential expression of both Runx2 and type 10 collagen [61].

Given the evidence for dysfunctional melatonin signalling in AIS osteoblasts, it is of particular interest that a recent study has implicated the involvement of melatonin in AIS growth plate endochondral ossification abnormalities [63]. That study reported that growth plate chondrocytes from AIS patients exhibited reduced expression of the melatonin receptor MT2 compared with normal growth plate chondrocytes; furthermore, in response to melatonin stimulation, there was no inhibitory effect on their proliferative activity. Given that the normal chondrocyte response to melatonin was inhibition of proliferation [63], the absence of this antiproliferative response to melatonin in AIS chondrocytes may result in driving a proliferative hypertrophic chondrocyte with an abnormal phenotype, which promotes endochondral ossification.

It is debatable whether these changes are primary events in the development of AIS. Indeed, animal models of idiopathic scoliosis suggest these changes may be a result of uneven loading [66]. Regardless, it is certainly feasible that a loss of proteoglycan matrix within the intervertebral disc or a differential change in chondrocyte phenotype between concave and convex vertebral growth plates could result in disc deformation and contribute towards promoting curve progression. Therefore, identifying candidate drivers of these abnormal changes in AIS intervertebral discs is important.

One family of candidate mediators are proinflammatory cytokines such as interleukin (IL)-1β, tumour necrosis factor (TNF)-α and IL-6, which are known drivers of cartilaginous matrix degeneration via the induction of matrix metalloproteases and aggrecanases [24]. Of potential significance, therefore, is a recent publication reporting a significant association between a functional polymorphism in IL-6 with susceptibility to idiopathic scoliosis and to curve severity [43]. Furthermore, there may be much to learn from studies that have examined the molecular basis for intervertebral disc degeneration in nonidiopathic scoliosis patient samples, where aberrant proliferation of cells in the nucleus pulposus is also implicated in the pathogenesis. Recent studies have reported that the noncoding micro-RNAs miR-10b and miR-21 can both promote nucleus pulposus cellular proliferation via inhibition of protein kinase B (AKT) signalling pathways [32, 67]. Both miR-10b and miR-21 expression were reported to be higher in nucleus pulposus tissue from patients with identified intervertebral disc degeneration compared with nucleus pulposus tissue from patients with idiopathic scoliosis. Importantly, however, it is not known how the expression of these miRNAs compares between idiopathic scoliosis and normal nondegenerative nucleus pulposus tissue.

Paraspinal muscles

Paraspinal (paravertebral) muscles play a key role in controlling spinal stability [13]. Therefore, one theory is that dysfunctional paraspinal muscles may contribute towards development of the scoliotic curve. Electromyography (EMG) analysis of paraspinal muscles to assess muscle activation patterns in AIS patients have provided evidence of asymmetry [2] and abnormalities in neuromuscular transmission [58]. It is not possible to determine from these particular studies whether such observations are secondary events. However, more recent studies have suggested that EMG activity of paraspinal muscles is predictive for curve progression in AIS patients [8, 11].

Several studies have reported differences in the paraspinal muscles between convex and concave sides of the curve in patients with AIS. In vivo measurements of muscle protein synthesis rates using stable isotopes have demonstrated reduced levels of protein synthesis in the paraspinal muscles on the concave side of the spinal curve compared with the convex paraspinal muscles [18]. Histological studies have also shown that paraspinal muscles on the concave side of the scoliosis apex exhibit greater fibrosis and fatty involution [60] compared with muscles on the convex side.

Few studies have compared the phenotype of paraspinal muscles from AIS patients with age-matched control paraspinal muscle tissue. One such study, however, found AIS concave paraspinal muscle exhibited a difference in the proportion of muscle fibre types, with a significantly lower percentage of type I slow fibres found in AIS compared with control muscle [38]. This finding suggests that a switch in the phenotype of AIS paraspinal muscle occurs, with a shift towards a faster, more glycolytic muscle phenotype, with reduced fatigue resistance. Of note, this particular study found no difference in fibre type between AIS and control muscles on the convex side, supporting the notion that it is the muscles on the concave side of the curve that are most affected in AIS [38].

These differential findings in paraspinal muscle pathology between concave and convex sides of the scoliosis apex could indicate that the muscle dysfunction is simply a secondary event due to postural changes upon spinal curvature. However, recent genetic studies have provided preliminary evidence that skeletal muscle dysfunction could be a contributory factor in AIS susceptibility: Firstly, rare variants in fibrillin-1 (FBN1) and fibrillin-2 (FBN2) have been found to be associated with severe AIS [6]. FBN1 and 2 are glycoproteins that form key components of skeletal muscle myofibrillar structure. Deficiency in FBN1 is associated with the connective tissue disorder Marfan's syndrome, a condition characterised by poor muscle development and muscle myopathy [4]. Indeed, 60 % of patients with Marfan's syndrome develop scoliosis. Interestingly, the recent publication by Wajchenberg et al. [60] reported signs of muscle myopathy and muscular atrophy in the paraspinal muscles on both the concave and convex sides of the scoliosis apex.

Similarly, a recent genome-wide association study identified the ladybird homeobox 1 (*LBX1*) locus as being associated with AIS susceptibility in both Asian and non-Hispanic

white populations [34]. From animal models, *LBX1* has been shown to play a critical role in muscular development. In addition, functional polymorphisms in the transforming growth factor beta (*TGF-β*) gene have been found to be associated with AIS susceptibility and in females to be associated with curve severity [53]. Abnormalities in TGF-β signalling are associated with several musculoskeletal disorders, including Duchenne muscular dystrophy [51], and it is a known inducer of muscle atrophy through induction in expression of muscle-specific ligase Atrogin-1 [19]. It is notable, therefore, that a recent study reported differential expression of TGF-β and TGF-β receptors with upregulation of *TGF-β*-responsive genes in paravertebral muscles from the concave side of the curve apex compared with the convex side [44]. This data is indicative of abnormal TGF-β signalling in the paravertebral muscles of patients with AIS and could therefore be a contributory factor to the abnormal paraspinal muscle pathology observed in AIS patients.

Epigenetics

It was initially envisaged that upon completion of the human genome project our understanding of human disease would lead to an abundance in the development of new targeted therapies by which to modify disease progression or prevent disease onset [25]. However, in recent years, it has become increasingly apparent that many diseases are likely to be the result of the interaction between intrinsic genes and the external environment [22], which can result in modification to gene transcription and thus impact biological processes. This understanding has led to the emergence of the research field known as epigenetics, which includes analysis of DNA methylation, histone modifications and transcription of noncoding RNAs [20, 27] such as miRNAs and long noncoding RNAs (lncRNAs). Such approaches may lead not only to an improved understanding of AIS but also help identify at-risk individuals and predict aggressive curves. Evidence that AIS may be a result of epigenetics is supported by reports of differences in spinal radiology in monozygotic twins with AIS [26] and from the increased incidence of AIS in relation to nutrition [64], physical activity [39] and maternal age [65]. Of importance, therefore, a recent study that performed microarray analysis and identified 139 lncRNAs (epigenetic regulators of gene transcription) that were differentially expressed in the peripheral blood of patients with AIS compared with control individuals [33]. As yet, however, no studies have performed RNA sequencing of AIS and control spinal human tissues to ascertain the full transcriptomic epigenetic profile of diseased AIS spinal tissue in humans. Furthermore, there are no reports of DNA methylation analysis of AIS patient tissues.

Summary

Despite AIS being the most common paediatric spinal deformity, very little is understood about the molecular basis of the disease pathology. However, current consensus is that AIS is likely to be a multifactorial condition involving both extrinsic and intrinsic factors, suggesting that epigenetic differences may underlie the disease pathology. To this end, it will be important to conduct epigenetic studies including full RNA transcriptomic sequencing and DNA methylation of spinal tissues (spinal bone, intervertebral disc, paravertebral muscles) in which AIS tissues are compared with age- and gender-matched normal control tissues. Ultimately, identifying the key intrinsic factors localised to the spinal tissues, and understanding their relationship to extrinsic factors and disease phenotype, may lead to the development of personalised disease-modifying therapeutics and also to biomarkers that can predict patient outcome, thus guiding clinical decision making.

Compliance with ethical standards

Conflict of interest None

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